

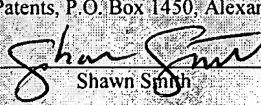
APPLICATION FOR UNITED STATES LETTERS PATENT

for

FULLERENE COMPOSITIONS FOR AMELIORATING  
DERMATOLOGICAL CONDITIONS

by

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The present application claims priority from U.S. Pat. Appl. 60/461,914, filed on April 10, 2003; U.S. Pat. Appl. 60/488,062, filed on July 17, 2003; U.S. Pat. Appl. 60/510,455, filed on October 10, 2003; U.S. Pat. Appl. 60/510,598, filed on October 10, 2003; and U.S. Pat. Appl. 60/510,283, filed on October 10, 2003. The disclosures of 5 each of the foregoing are incorporated herein by reference.

### BACKGROUND OF THE INVENTION

10 The present invention relates generally to the fields of ameliorating dermatological conditions. More particularly, it concerns the use of fullerene compositions to treat dermatological conditions such as sunburn, aging, and the like.

15 Reactive oxygen species (ROS), commonly referred to as “free radicals,” have been implicated in a variety of dermatological conditions, including, but not limited to, sunburn, aging, and smoker’s face. ROS are believed to promote, in at least certain cells, cell types, tissues, or tissue types of the skin, cell death (apoptosis), impaired cellular function, and modification or change in proportion of extracellular matrix components such as elastin or collagen, among other symptoms.

A need exists to treat dermatological conditions by administering compounds that can neutralize ROS.

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### SUMMARY OF THE INVENTION

25 In one embodiment, the present invention relates to administering a composition comprising a substituted fullerene to at least a portion of the skin of the mammal afflicted with the dermatological condition or potentially afflicted with the dermatological condition, wherein the substituted fullerene comprises a fullerene core (C<sub>n</sub>) and at least one of:

- (i) from 1 to 3 (>C<sup>1</sup>X<sup>2</sup>) groups bonded to the fullerene core;
- (ii) from 1 to 18 -X<sup>3</sup> groups bonded to the fullerene core;
- 30 (iii) from 1 to 6 -X<sup>4</sup>- groups bonded to the fullerene core; or

(iv) from 1 to 6 dendrons bonded to the fullerene core.

Herein, the word "or" has the inclusive meaning wherever it appears.

In another embodiment, the present invention relates to a composition for ameliorating a dermatological condition in the skin of a mammal, comprising (a) a substituted fullerene; and (b) a pharmaceutically acceptable carrier.

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#### BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to 10 further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Figure 1 shows (day 0) two portions (inside the box and outside the box) of the 15 skin of a human test subject shortly after several hours of exposure to daytime sunlight at about 22° north latitude and immediately before commencing an application regimen of a composition comprising a substituted fullerene to the portion inside the box and (day 2) the same two portions of the skin about 48 hr after completion of the application regimen.

Figure 2A shows an exemplary substituted fullerene in structural formula, and Figure 2B shows the same substituted fullerene in a schematic formula.

20 Figure 3 shows the decarboxylation of C3 to C3-penta-acid and thence to C3-tetra-acid.

Figure 4 shows the decarboxylation of C3-tetra-acid to C3-tris-acid.

Figure 5 shows the chirality of C3.

Figure 6 shows the effect of C3 chirality on isomers formed by decarboxylation.

25 Figure 7 shows exemplary substituted fullerenes according to one embodiment of the present invention. C3, in the upper left, is comparative.

Figures 8A and 8B show two exemplary substituted fullerenes.

Figures 9A-9G show seven exemplary dendrofullerenes.

Figure 10 shows dendrofullerene DF-1.

Figures 11A-11H report the IC<sub>50</sub> values for various substituted fullerenes (and Trolox, a known non-fullerene antioxidant), as described in Example 2.

### DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

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In one embodiment, the present invention relates to a method of ameliorating a dermatological condition in the skin of a mammal, comprising:

10 administering a composition comprising a substituted fullerene to at least a portion of the skin of the mammal afflicted with the dermatological condition or potentially afflicted with the dermatological condition.

By “ameliorating” a disease is meant improving the condition of a subject suffering or at risk of suffering from the disease. Ameliorating can comprise one or more of the following: a reduction in the severity of a symptom of the disease, a reduction in the extent of a symptom of the disease, a reduction in the number of symptoms of the disease, a reduction in the number of disease agents, a reduction in the spread of a symptom of the disease, a delay in the onset of a symptom of the disease, a delay in disease onset, or a reduction in the time between onset of the disease and remission of the disease, among others apparent to the skilled artisan having the benefit of the present disclosure. To the extent that the foregoing examples of ameliorating a disease are defined in relative terms, the proper comparison is to the disease or symptoms thereof when no composition is administered to ameliorate it and no method is performed to ameliorate it. The terms “preventing” (herein meaning “to stop a disease from onsetting”) and “treating” (herein meaning “to improve the condition of a mammal suffering from a disease”) are both within the scope of “ameliorating,” as used herein.

25 In the present invention, the disease is an oxidative stress disease. An “oxidative stress disease” is a disease in which the healthy function of one or more organelles, non-organelle subcellular structures, cells, cell types, tissues, tissue types, organs, or organ systems is impaired by the action of oxidizing agents, such as free radicals, particularly radical oxygen species (ROS). The action of oxidizing agents need not be the only route 30 by which impairment of healthy function occurs in the course of a disease for the disease

to be an oxidative stress disease. In oxidative stress diseases, a number of sources of oxidizing agents are known. Exemplary sources include, but are not limited to, by-processes of metabolism, irritation by environmental agents (for example, tobacco smoke or ultraviolet light), or internal challenge (for example, ischemia), among others.

5 Any one or more of a large number of oxidative stress diseases can be ameliorated by performance of the method.

A “dermatological condition,” as the term is used herein, refers to an ailment which afflicts at least a portion of the skin of a mammal. By “afflicts at least a portion of the skin” is meant that all or part of the skin of the mammal experiences one or more of 10 cell death, cell injury, impaired cell function, the production of cellular products reflective of cell injury, the proliferation of cell types not normally present in the skin or not normally present in the skin at such high levels, the degradation or alteration of extracellular matrix, or other symptoms generally recognizable by the skilled artisan as indicating a dermatological condition when seen in one or more cells, cell types, tissues, 15 or tissue types of skin.

Exemplary causes of dermatological conditions include, but are not limited to, flame, heat, and radiation, such as alpha particles, beta particles, or electromagnetic radiation, such as ultraviolet light (UV) or gamma rays, among others.

In one embodiment, the dermatological condition is sunburn. The term “sunburn” 20 is intended to cover any damage inflicted on skin by one or more of electromagnetic radiation of any wavelength or wavelengths, alpha radiation, or beta radiation, regardless whether the source of the radiation is the sun or another star. In another embodiment, the dermatological condition is aging. “Aging,” as used herein, encompasses both changes in the external morphology of the skin associated with the passage of time (e.g., the 25 formation of laugh lines, crow’s feet, or other wrinkles; or the emergence of liver spots) and molecular changes underlying the above changes in the external morphology (e.g., changes in concentration of elastin, collagen, or both in the extracellular matrix of skin). In yet another embodiment, the dermatological condition is psoriasis. In still another embodiment, the dermatological condition is acne. In a further embodiment, the

dermatological condition is smoker's face. In yet another embodiment, the dermatological condition is sunburn, aging, hair loss, or smoker's face.

In one embodiment, the oxidative stress disease is senescence. "Senescence," as used herein, refers to one or more of a decrease in the overall health of a mammal, a 5 decrease in the overall fitness of a mammal, or a decrease in the overall quality of life of a mammal, wherein such decrease is generally attributed to the aging process. In one embodiment, ameliorating senescence may lead to maintenance of a particular level of systemic well-being to a later point in the mammal's life. In one embodiment, ameliorating senescence may lead to at least a partial increase in the expected lifespan of 10 the mammal.

Methods of enhancing the overall health and longevity of humans and their 15 companions has been a very active area of research. Given the conserved nature of cellular or developmental processes across metazoans, a number of model organisms have been employed to study senescence, including a nematode, *Caenorhabditis elegans*, and a fruit fly, *Drosophila melanogaster*.

For example, the genetic analysis of *C. elegans* has revealed several genes 20 involved in lifespan determination. Mutations in Daf-2 (an insulin receptor) and Clk-1 ("Clock 1", a gene affecting many aspects of developmental and behavioral timing) have been shown to extend the lifespan of *C. elegans* adults. However, Clk-1 mutants have a higher mortality rate in early life. The Clk-1 longevity phenotype is abolished by 25 mutations in the gene encoding catalase, which is involved in superoxide/free radical metabolism. Additionally, elimination of coenzyme Q in *C. elegans* diet has been shown to extend lifespan. These observations suggest reactive oxygen species are involved in senescence in *C. elegans*.

In *Drosophila*, superoxide dismutase (SOD) and catalase overexpression 30 increased the lifespan by 35%. Mutations in the Methuselah gene ("Mth") have been shown to increase lifespan by 20%. The function of Mth, a G-protein coupled receptor, is not known, but mutants have shown an increased resistance to paraquat (a superoxide radical injury inducing agent). These observations suggest reactive oxygen species are involved in senescence in *Drosophila*.

Dugan *et al.*, Publ. Patent Appl. US 2003/0162837, reported the oral administration of C3 to mice (at about 0.5 mg/kg/day) led to about a 20% increase in mean survival relative to controls (28.7 +/- 3.3 months vs. 23.5 +/- 5.5 months, p = 0.033), thus suggesting an antioxidant compound was capable of ameliorating

5 senescence.

By "skin" is meant both the general integument providing the majority of the encasement of a mammalian body and specialized tissues forming hair, mucosae, the papillae of the breast, or the integument of the mouth, the genitals, the nails, the hooves, or the claws, among others apparent to one of ordinary skill in the art. Any quantity of *in vivo* skin, ranging in surface area from about 1 mm<sup>2</sup> to the entirety of the mammal's skin surface, can be considered a "portion" as this term is used in the description and claims.

10 Any mammal which suffers a dermatological condition, as defined herein, can receive the administered composition. An exemplary mammal is *Homo sapiens*, although other mammals possessing economic or esthetic utility (e.g., livestock such as cattle, sheep, or horses; e.g., pets such as dogs and cats) can receive the administered 15 composition.

20 The term "administering," as used herein, is intended to encompass all techniques of introducing a composition to at least a portion of the skin of a mammal. Exemplary routes of administration include topical application and subcutaneous injection, among others, including systemic routes of administration such as intravenous, intraarterial, intramuscular, oral, rectal, and nasal, among others.

The composition comprises a substituted fullerene.

25 Buckminsterfullerenes, also known as fullerenes or, more colloquially, buckyballs, are cage-like molecules consisting essentially of sp<sup>2</sup>-hybridized carbons and have the general formula (C<sub>20+2m</sub>) (where m is a natural number). Fullerenes are the third form of pure carbon, in addition to diamond and graphite. Typically, fullerenes are arranged in hexagons, pentagons, or both. Most known fullerenes have 12 pentagons and varying numbers of hexagons depending on the size of the molecule. "C<sub>n</sub>" refers to a fullerene moiety comprising n carbon atoms.

Common fullerenes include C<sub>60</sub> and C<sub>70</sub>, although fullerenes comprising up to about 400 carbon atoms are also known.

Fullerenes can be produced by any known technique, including, but not limited to, high temperature vaporization of graphite. Fullerenes are available from MER 5 Corporation (Tucson, AZ) and Frontier Carbon Corporation, among other sources.

A substituted fullerene is a fullerene having at least one substituent group bonded to at least one carbon of the fullerene core. Exemplary substituted fullerenes include carboxyfullerenes and hydroxylated fullerenes, among others.

A carboxyfullerene, as used herein, is a fullerene derivative comprising a C<sub>n</sub> core 10 and one or more substituent groups, wherein at least one substituent group comprises a carboxylic acid moiety or an ester moiety. Generally, carboxyfullerenes are water soluble, although whether a specific carboxyfullerene is water soluble is a matter of routine experimentation for the skilled artisan.

In another embodiment, the fullerene is a hydroxylated fullerene. A 15 “hydroxylated fullerene,” as used herein, is a fullerene derivative comprising a C<sub>n</sub> core and one or more substituent groups, wherein at least one substituent group comprises a hydroxyl moiety.

In all embodiments, the substituted fullerene comprises a fullerene core (C<sub>n</sub>), 20 which can have any number of carbon atoms. In one embodiment, the C<sub>n</sub> has 60 carbon atoms (and may be represented herein as C<sub>60</sub>). In one embodiment, the C<sub>n</sub> has 70 carbon atoms (and may be represented herein as C<sub>70</sub>).

Throughout this description, particular embodiments described herein may be 25 described in terms of a particular acid, amide, ester, or salt conformation, but the skilled artisan will understand an embodiment can change among these and other conformations depending on the pH and other conditions of manufacture, storage, and use. All such conformations are within the scope of the appended claims. The conformational change between, *e.g.*, an acid and a salt is a routine change, whereas a structural change, such as the decarboxylation of an acid moiety to -H, is not a routine change.

In one embodiment, the substituted fullerene comprises a fullerene core (C<sub>n</sub>) and 30 from 1 to 3 (>C<sup>1</sup>X<sup>2</sup>) groups bonded to the fullerene core. The notation “>C” indicates

the group is bonded to the fullerene core by two single bonds between the carbon atom "C" and the Cn. X<sup>1</sup> and X<sup>2</sup> can be, independently, any moiety containing at least one carbon atom, wherein, when the substituted fullerene consists of a fullerene core and one or more (>CX<sup>1</sup>X<sup>2</sup>) groups bonded thereto, at least one X<sup>1</sup> or X<sup>2</sup> moiety of at least one (>CX<sup>1</sup>X<sup>2</sup>) group is not -COOH or the (>CX<sup>1</sup>X<sup>2</sup>) groups are not in the C3 or D3 orientation on the fullerene core.

5 In one further embodiment, X<sup>1</sup> can be selected from -H, -COOH, -CONH<sub>2</sub>, -CONR', -CONR'<sub>2</sub>, -COOR', -CHO, -(CH<sub>2</sub>)<sub>d</sub>OH, or a salt thereof, wherein each R' is independently (i) a hydrocarbon moiety having from 1 to about 6 carbon atoms or (ii) a 10 hydrocarbon moiety having from 1 to about 6 carbon atoms and a terminal carboxylic acid, and d is an integer from 0 to about 20. In another further embodiment, X<sup>1</sup> can be selected from -R, -RCOOH, -RCONH<sub>2</sub>, -RCONR', -RCONR'<sub>2</sub>, -RCOOR', -RCHO, -R(CH<sub>2</sub>)<sub>d</sub>OH, or a salt thereof, wherein R is a hydrocarbon moiety having from 1 to about 6 carbon atoms.

15 Similarly, but independently, in one embodiment X<sup>2</sup> can be selected from -H, -COOH, -CONH<sub>2</sub>, -CONR', -CONR'<sub>2</sub>, -COOR', -CHO, -(CH<sub>2</sub>)<sub>d</sub>OH, or a salt thereof. In another embodiment, X<sup>2</sup> can be selected from -R, -RCOOH, -RCONH<sub>2</sub>, -RCONR', -RCONR'<sub>2</sub>, -RCOOR', -RCHO, -R(CH<sub>2</sub>)<sub>d</sub>OH, or a salt thereof.

20 All structural formulas shown herein are not to be construed as limiting the structure to any particular isomer.

25 All possible isomers of the substituted fullerenes disclosed herein are within the scope of the present disclosure. For example, in >CX<sup>1</sup>X<sup>2</sup>, one group (X<sup>1</sup> or X<sup>2</sup>) of each substituent points away from the fullerene core, and the other group points toward the fullerene core. Continuing the example, the central carbon of each substituent group (by which is meant the carbon with two bonds to the fullerene core, one bond to X<sup>1</sup>, and one bond to X<sup>2</sup>) is chiral when X<sup>1</sup> and X<sup>2</sup> are different.

It will also be apparent that substituted fullerenes having two or more substituent groups will have isomers resulting from the different possible sites of bonding of the substituent groups to the fullerene core.

In one embodiment, the substituted fullerene is a decarboxylation product of (C<sub>60</sub>(>C(COOH)<sub>2</sub>)<sub>3</sub>) (C3). By “decarboxylation product of C3” is meant the product of a reaction wherein 0 or 1 carboxy (-COOH) groups are removed from each of the three malonate moieties (>C(COOH)<sub>2</sub>) of C3 and replaced with -H, provided at least one of the 5 malonate moieties has 1 carboxy group replaced with -H. This can be considered as the loss of CO<sub>2</sub> from a malonate moiety. Decarboxylation can be performed by heating C3 in acid, among other techniques.

During decarboxylation of C3, only CO<sub>2</sub> loss from C3 is observed; each malonate 10 moiety retains at least one carboxyls; and the decarboxylation stops at loss of 3 CO<sub>2</sub> groups, one from each malonate moiety of C3. The skilled artisan having the benefit of the present disclosure will recognize that substituted fullerenes having 1, 2, 4, 5, or 6 malonate moieties would also undergo decarboxylation.

In C3, each malonate moiety has a carboxy group pointing to the outside (away 15 from the fullerene), which we herein term *exo*, and a carboxy group pointing to the inside (toward the fullerene), which we herein term *endo*. Figure 2A presents both a structural formula of C3 (Figure 2A) and a diagram representing *exo*- and *endo*-acids (Figure 2B).

Figure 3 shows C3 (in box 30) and the products of subsequent loss via 20 decarboxylation of one or two CO<sub>2</sub> groups, giving C3-penta-acids (in box 32) and C3-tetra-acids (in box 34). Decarboxylation is represented by the open arrows 31 and 33; the isomers of C3, C3-penta-acid, and C3-tetra-acid are shown in box 30, in box 32, and in box 34, respectively.

In the interest of precise nomenclature, we define the order of *exo* or *endo* by 25 always naming the groups in a clockwise manner.

Figure 4 shows the products of subsequent loss via decarboxylation of a third CO<sub>2</sub> group from the C3-tetra-acids shown in box 34, giving C3-tris-acids (box 42). Decarboxylation is represented by the open arrow 41; the isomers of C3-tetra-acid and C3-penta-acid are shown in box 34 and in box 42, respectively. Isomers that differ only by rotation are connected by dashed lines 43, 44, and 45..

Figure 5 shows the chirality of C3, both in a structural formula (mirror images 30 50a and 50b) and a schematic representation (mirror images 52a and 52b). Figure 6

shows the chirality of C3-penta-acids (mirror images 60a and 60b; mirror images 62a and 62b).

In another embodiment, the substituted fullerene comprises one of the structures 72, 74, 76, 77, or 78 shown in Figure 7.

5 In one embodiment, the substituted fullerene comprises C<sub>60</sub> and 3 (>CX<sup>1</sup>X<sup>2</sup>) groups in the C3 orientation or the D3 orientation. The D3 orientation is a mirror translation of the C3 orientation. The above description of C3-penta-acids, C3-tetra-acids, and C3-tris-acids also applies to D3 orientations of penta acids, tetra acids, and tris acids.

10 In one embodiment, as shown in Figure 11, the substituted fullerene comprises C<sub>60</sub> and 2 (>CX<sup>1</sup>X<sup>2</sup>) groups in the trans-2 orientation 1206, the trans-3 orientation 1207, the e orientation 1208, or the cis-2 orientation 1209.

In another embodiment, also as shown in Figure 11, the substituted fullerene comprises C<sub>70</sub> and 2 (>CX<sup>1</sup>X<sup>2</sup>) groups in the bis orientation 1210 or 1211.

15 In one embodiment, the substituted fullerene has the formula C<sub>60</sub>(>C(COOR)<sub>2</sub>)<sub>n</sub>, wherein -R is -H or an organic moiety comprising one or more carbon atoms, and n is an integer from 1 to 30. The -R moiety can be bonded to another -R moiety of the same or another substituent group. In another embodiment, the substituted fullerene has the structure shown in Figure 8B.

20 In one embodiment, the substituted fullerene comprises a fullerene core (C<sub>n</sub>) and from 1 to 18 -X<sup>3</sup> groups bonded to the fullerene core. The notation “-X<sup>3</sup>” indicates the group is bonded to the fullerene core by a single bond between one atom of the X<sup>3</sup> group and one carbon atom of the fullerene core. In specific X<sup>3</sup> groups referred to below, any unfilled valences represent the single bond between the group and the fullerene core.

25 In one embodiment, the substituted fullerene comprises from 1 to about 6 -X<sup>3</sup> groups and each -X<sup>3</sup> group is independently selected from:

-N<sup>+</sup>(R<sup>2</sup>)(R<sup>3</sup>)(R<sup>4</sup>), wherein R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> are independently -H or -(CH<sub>2</sub>)<sub>d</sub>-CH<sub>3</sub>, wherein d is an integer from 0 to about 20;

-N(R<sup>2</sup>)(R<sup>3</sup>)(R<sup>8</sup>), wherein R<sup>2</sup> and R<sup>3</sup> are independently -H or -(CH<sub>2</sub>)<sub>d</sub>-CH<sub>3</sub>, wherein d is an integer from 0 to about 20, and each R<sup>8</sup> is independently -(CH<sub>2</sub>)<sub>f</sub>SO<sub>3</sub><sup>-</sup>,

-(CH<sub>2</sub>)<sub>f</sub>PO<sub>4</sub><sup>-</sup>, or -(CH<sub>2</sub>)<sub>f</sub>COO<sup>-</sup>, wherein f is an integer from 1 to about 20;

-C(R<sup>5</sup>)(R<sup>6</sup>)(R<sup>7</sup>), wherein R<sup>5</sup>, R<sup>6</sup>, and R<sup>7</sup> are independently -COOH, -H, -CH(=O),  
5 or -CH<sub>2</sub>OH;

-C(R<sup>2</sup>)(R<sup>3</sup>)(R<sup>8</sup>), wherein R<sup>2</sup> and R<sup>3</sup> are independently -H or -(CH<sub>2</sub>)<sub>d</sub>-CH<sub>3</sub>, wherein d is an integer from 0 to about 20, and each R<sup>8</sup> is independently -(CH<sub>2</sub>)<sub>f</sub>SO<sub>3</sub><sup>-</sup>,

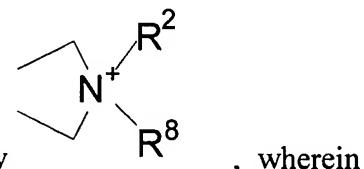
-(CH<sub>2</sub>)<sub>f</sub>PO<sub>4</sub><sup>-</sup>, or -(CH<sub>2</sub>)<sub>f</sub>COO<sup>-</sup>, wherein f is an integer from 1 to about 20;

10 -(CH<sub>2</sub>)<sub>e</sub>-COOH, -(CH<sub>2</sub>)<sub>e</sub>-CONH<sub>2</sub>, -(CH<sub>2</sub>)<sub>e</sub>-COOR', or a peptidyl moiety, wherein e is an integer from 1 to about 6 and each R' is independently (i) a hydrocarbon moiety having from 1 to about 6 carbon atoms or (ii) a hydrocarbon moiety having from 1 to about 6 carbon atoms and a terminal carboxylic acid; or

an aromatic heterocyclic moiety containing a cationic nitrogen.

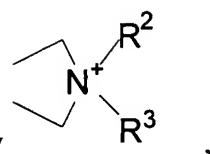
As will be apparent from the foregoing, a substituted fullerene according to this embodiment can comprise one or more groups selected from one or more of the foregoing categories.

In another embodiment, the substituted fullerene comprises a fullerene core (C<sub>n</sub>) and from 1 to 6 -X<sup>4</sup>- groups bonded to the fullerene core. The notation “-X<sup>4</sup>-” indicates the group is bonded to the fullerene core by two single bonds, wherein one single bond is between a first atom of the group and a first carbon of the fullerene core, and the other single bond is between a second atom of the group and a second carbon of the fullerene core. (The adjectives “first” and “second,” wherever they appear herein, do not imply a particular ordering, in time, space, or both, of the nouns modified by the adjectives).



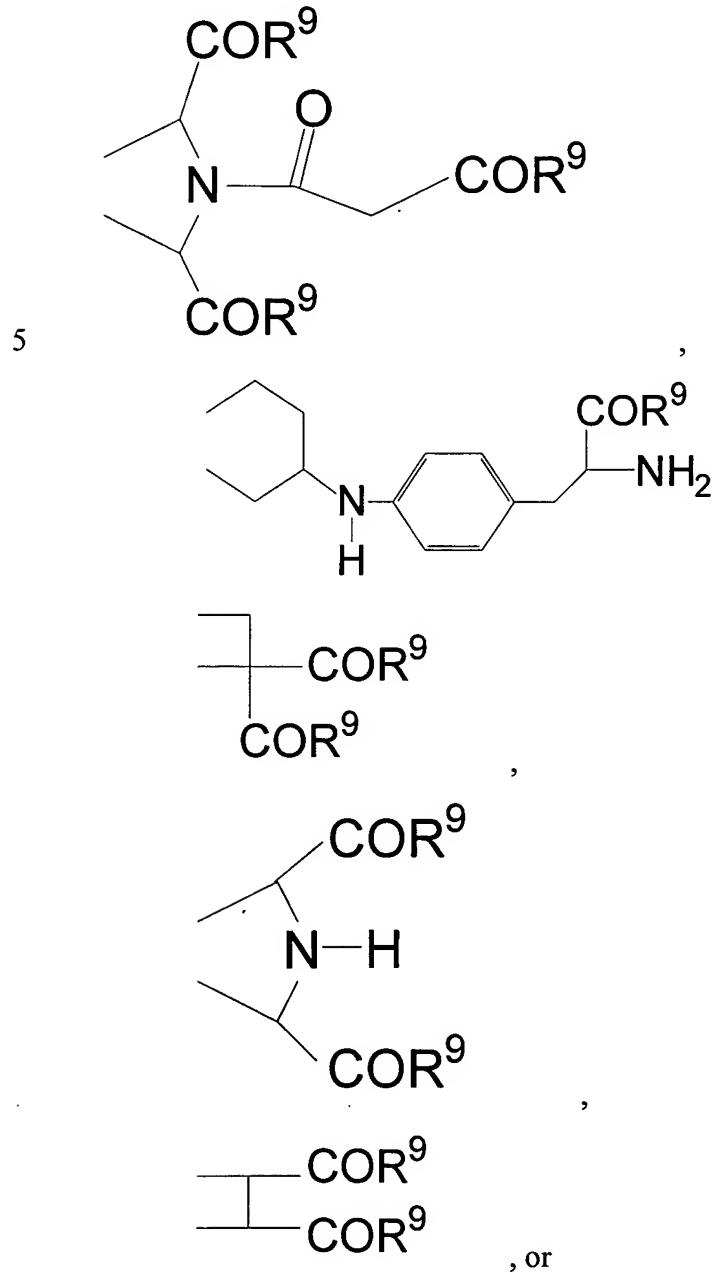
In one embodiment, each -X<sup>4</sup>- group is independently

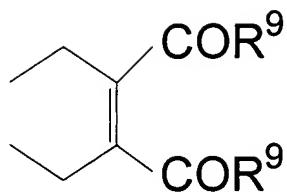
25 R<sup>2</sup> is independently -H or -(CH<sub>2</sub>)<sub>d</sub>-CH<sub>3</sub>, wherein d is an integer from 0 to about 20, and R<sup>8</sup> is independently -(CH<sub>2</sub>)<sub>f</sub>SO<sub>3</sub><sup>-</sup>, -(CH<sub>2</sub>)<sub>f</sub>PO<sub>4</sub><sup>-</sup>, or -(CH<sub>2</sub>)<sub>f</sub>COO<sup>-</sup>, wherein f is an integer from 1 to about 20.



In another embodiment, each  $-X^4-$  group is independently  $\text{R}^3$ , wherein each  $\text{R}^2$  and  $\text{R}^3$  is independently -H or  $-(\text{CH}_2)_d-\text{CH}_3$ , wherein d is an integer from 0 to about 20.

In another embodiment, each  $-X^4-$  group is independently selected from:





wherein each  $\text{R}^2$  is independently -H or  $-(\text{CH}_2)_d\text{CH}_3$ , wherein d is an integer from 0 to about 20, and each  $\text{R}^9$  is independently -H, -OH, -OR', -NH<sub>2</sub>, -NHR', -NHR'<sub>2</sub>, or  $-(\text{CH}_2)_d\text{OH}$ , wherein each R' is independently (i) a hydrocarbon moiety having from 1 to about 6 carbon atoms or (ii) a hydrocarbon moiety having from 1 to about 6 carbon atoms and a terminal carboxylic acid.

In one embodiment of the present invention, the substituted fullerene comprises a fullerene core ( $\text{C}_n$ ) and from 1 to 6 dendrons bonded to the fullerene core.

A dendron within the meaning of the invention is an addendum of the fullerene which has a branching at the end as a structural unit. Dendrons can be considered to be derived from a core, wherein the core contains two or more reactive sites. Each reactive site of the core can be considered to have been reacted with a molecule comprising an active site (in this context, a site that reacts with the reactive site of the core) and two or more reactive sites, to define a first generation dendron. First generation dendrons are within the scope of the term "dendron," as used herein. Higher generation dendrons can be considered to have formed by each reactive site of the first generation dendron having been reacted with the same or another molecule comprising an active site and two or more reactive sites, to define a second generation dendron, with subsequent generations being considered to have been formed by similar additions to the latest generation.

Although dendrons can be formed by the techniques described above, dendrons formed by other techniques are within the scope of "dendron" as used herein.

The core of the dendron is bonded to the fullerene by one or more bonds between (a) one or more carbons of the fullerene and (b) one or more atoms of the core. In one embodiment, the core of the dendron is bonded to the fullerene in such a manner as to form a cyclopropyl ring.

In one embodiment, the core of the dendron comprises, between the sites of binding to the fullerene and the reactive sites of the core, a spacer, which can be a chain of 1 to about 100 atoms, such as about 2 to about 10 carbon atoms.

5 The generations of the dendron can comprise trivalent or polyvalent elements such as, for example, N, C, P, Si, or polyvalent molecular segments such as aryl or heteroaryl. The number of reactive sites for each generation can be about two or about 10 three. The number of generations can be between 1 and about 10, inclusive.

10 More information regarding dendrons suitable for adding to fullerenes can be found in Hirsch, U.S. Pat. No. 6,506,928, the disclosure of which is hereby incorporated by reference.

15 In a further embodiment, the substituted fullerene has a structure selected from Figures 9A-9G. In Figure 9D, each “Sugar” independently represents a carbohydrate moiety, and each “linker” independently represents an organic or inorganic moiety. In a further embodiment, each Sugar is independently ribose or deoxyribose, and each 20 “linker” independently has the formula  $-(CH_2)_d-$ , wherein d is an integer from 0 to about 20.

20 The substituted fullerene of this embodiment can further comprise a nondendron moiety, which is an addendum to a fullerene, wherein the addendum does not have a core and generations structure as found in dendrons defined above. Exemplary nondendrons include, but are not limited to, -COOH, -When the dendron comprises 18 -COOH groups, the substituted fullerene comprises one or more nondendrons.

The substituted fullerene of the present invention can satisfy one, two, or more of the foregoing embodiments, consistent with the plain meaning of “comprising.”

25 A substituted fullerene of any of the foregoing embodiments can further comprise an endohedral metal. “Metal” means at least one atom of a metallic element, and “endohedral” means the metal is engaged by the fullerene core. The metal can be elemental, or it can be an atom or atoms in a molecule comprising other elements. A substituted fullerene comprising an endohedral metal can be termed a “metallofullerene.” In a further embodiment, the metallofullerene can be represented by the structure:

30  $M_m @ C_n$ ,

wherein each M independently is a molecule containing a metal;  
m is an integer from 1 to about 5; and  
C<sub>n</sub> is a fullerene core comprising n carbon atoms, wherein n is an integer equal to or greater than 60.

5 In one embodiment, M is a transition metal atom. In one embodiment, M is a metal atom with an atomic number greater than about 55. Exemplary metals include those which do not form metal carbides. In one embodiment, the metal is Ho, Gd, or Lu.

10 In one embodiment, M is an organometallic molecule or an inorganometallic molecule. In one embodiment, M is a molecule having the formula M'3N, wherein each M' independently is a metal atom. Each metal atom M' can be any metal, such as a transition metal, a metal with an atomic number greater than about 55, or one of the exemplary metals given above, among others.

In one embodiment, M is a metal capable of reacting with a reactive oxygen species.

15 In one embodiment, the metallofullerene is characterized in that M is Ho, Ho<sub>3</sub>N, Gd, Gd<sub>3</sub>N, Lu, or Lu<sub>3</sub>N; m is 1; and n is 60.

20 In one embodiment, the substituted fullerene is polymerized, by which is meant a plurality of fullerene cores are present in a single molecule. The molecule can comprise carbon-carbon bonds between a first fullerene core and a second fullerene core, covalent bonds between a first substituent group on a first fullerene core and a second substituent group on a second fullerene core, or both.

25 The substituted fullerene can be used in the method as a composition with other components. In one embodiment, the composition further comprises an amphiphilic fullerene having the formula (B)<sub>b</sub>-C<sub>n</sub>-(A)<sub>a</sub>, wherein C<sub>n</sub> is a fullerene moiety comprising n carbon atoms, wherein n is an integer and 60 ≤ n ≤ 240; B is an organic moiety comprising from 1 to about 40 polar headgroup moieties; b is an integer and 1 ≤ b ≤ 5; each B is covalently bonded to the C<sub>n</sub> through 1 or 2 carbon-carbon, carbon-oxygen, or carbon-nitrogen bonds; A is an organic moiety comprising a terminus proximal to the C<sub>n</sub> and one or more termini distal to the C<sub>n</sub>, wherein the termini distal to the C<sub>n</sub> each 30 comprise -C<sub>x</sub>H<sub>y</sub>, wherein x is an integer and 8 ≤ x ≤ 24, and y is an integer and 1 ≤ y ≤

2x+1; a is an integer,  $1 \leq a \leq 5$ ;  $2 \leq b+a \leq 6$ ; and each A is covalently bonded to the  $C_n$  through 1 or 2 carbon-carbon, carbon-oxygen, or carbon-nitrogen bonds.

B is chosen from any organic moiety comprising from 1 to about 40 polar headgroup moieties. A “polar headgroup” is a moiety which is polar, by which is meant that the vector sum of the bond dipoles of each bond within the moiety is nonzero. A polar headgroup can be positively charged, negatively charged, or neutral. The polar headgroup can be located such that at least a portion of the moiety can be exposed to the environment of the molecule. Exemplary polar headgroup moieties can include, but are not limited to, carboxylic acid, alcohol, amide, and amine moieties, among others known in the art. Preferably, B has from about 6 to about 24 polar headgroup moieties. In one embodiment, B has a structure wherein the majority of the polar headgroup moieties are carboxylic acid moieties, which are exposed to water when the amphiphilic fullerene is dissolved in an aqueous solvent. A dendrimeric or other regular highly-branched structure is suitable for the structure of B.

The value of b can be any integer from 1 to 5. In one embodiment, if more than one B group is present (i.e.,  $b > 1$ ), that all such B groups are adjacent to each other. By “adjacent” in this context is meant that no B group has only A groups, as defined below, and/or no substituent groups at all the nearest neighboring points of addition. In the case of an octahedral addition pattern when  $b > 1$ , “adjacent” means that the four vertices of the octahedron in closest proximity to the B group are not all A groups or null.

In one embodiment, B comprises 18 polar headgroup moieties and  $b = 1$ .

The polar headgroup moieties of B tend to make the B group or groups hydrophilic.

Each B is bonded to  $C_n$  through a covalent bond or bonds. Any covalent bond which a fullerene carbon is capable of forming and will not disrupt the fullerene structure is contemplated. Examples include carbon-carbon, carbon-oxygen, or carbon-nitrogen bonds. One or more atoms, such as one or two atoms, of the B group can participate in bonding to  $C_n$ . In one embodiment, one carbon atom of the B group is bonded to two carbon atoms of  $C_n$ , wherein the two carbon atoms of  $C_n$  are bonded to each other.

In one embodiment, B has the amide dendron structure

>C(C(=O)OC<sub>3</sub>H<sub>6</sub>C(=O)NHC(C<sub>2</sub>H<sub>4</sub>C(=O)NHC(C<sub>2</sub>H<sub>4</sub>C(=O)OH)<sub>3</sub>)<sub>3</sub>)<sub>2</sub>.

In the amphiphilic fullerene, A is an organic moiety comprising a terminus proximal to the C<sub>n</sub> and one or more termini distal to the C<sub>n</sub>. In one embodiment, the organic moiety comprises two termini distal to C<sub>n</sub>. By “terminus proximal to C<sub>n</sub>” is meant a portion of the A group that comprises one or more atoms, such as one or two atoms, of the A group which form a bond or bonds to C<sub>n</sub>. By “terminus distal to C<sub>n</sub>” is meant a portion of the A group that does not comprise any atoms which form a bond or bonds to C<sub>n</sub>, but that does comprise one or more atoms which form a bond or bonds to the terminus of the A group proximal to C<sub>n</sub>.

10 Each terminus distal to the C<sub>n</sub> comprises -C<sub>x</sub>H<sub>y</sub>, wherein x is an integer and 8 ≤ x ≤ 24, and y is an integer and 1 ≤ y ≤ 2x+1. The -C<sub>x</sub>H<sub>y</sub> can be linear, branched, cyclic, aromatic, or some combination thereof. Preferably, A comprises two termini distal to C<sub>n</sub>, wherein each -C<sub>x</sub>H<sub>y</sub> is linear, 12 ≤ x ≤ 18, and y = 2x+1. More preferably, in each of the two termini, x = 12 and y = 25.

15 The termini distal to C<sub>n</sub> tend to make the A groups hydrophobic or lipophilic.

The value of a can be any integer from 1 to 5. Preferably, a is 5. In one embodiment, if more than one A group is present (i.e., a > 1), all such A groups are adjacent to each other. By “adjacent” in this context is meant that no A group has only B groups, as defined below, and/or no substituent groups at all the nearest neighboring 20 points of addition. In the case of an octahedral addition pattern, when a > 1, “adjacent” means that the four vertices of the octahedron in closest proximity to the A group are not all B groups or null.

25 Each A is bonded to C<sub>n</sub> through a covalent bond or bonds. Any covalent bond which a fullerene carbon is capable of forming and will not disrupt the fullerene structure is contemplated. Examples include carbon-carbon, carbon-oxygen, or carbon-nitrogen bonds. One or more atoms, such as one or two atoms, of the A group can participate in bonding to C<sub>n</sub>. In one embodiment, one carbon atom of the A group is bonded to two carbon atoms of C<sub>n</sub>, wherein the two carbon atoms of C<sub>n</sub> are bonded to each other.

In one embodiment, A has the structure >C(C(=O)O(CH<sub>2</sub>)<sub>11</sub>CH<sub>3</sub>)<sub>2</sub>.

The number of B and A groups is chosen to be from 2 to 6, i.e.,  $2 \leq b+a \leq 6$ . In one embodiment,  $b+a = 6$ . The combination of hydrophilic B group(s) and hydrophobic A group(s) renders the fullerene amphiphilic. The number and identity of B groups and A groups can be chosen to produce a fullerene with particular amphiphilic qualities  
5 which may be desirable for particular intended uses.

The amphiphilic fullerenes are capable of forming a vesicle, wherein the vesicle wall comprises the amphiphilic fullerene. A “vesicle,” as the term is used herein, is a collection of amphiphilic molecules, by which is meant, molecules which include both (a) hydrophilic (“water-loving”) regions, typically charged or polar moieties, such as  
10 moieties comprising polar headgroups, among others known to one of ordinary skill in the art, and (b) hydrophobic (“water-hating”) regions, typically apolar moieties, such as hydrocarbon chains, among others known to one of ordinary skill in the art. In aqueous solution, the vesicle is formed when the amphiphilic molecules form a wall, i.e., a closed three-dimensional surface. The wall defines an interior of the vesicle and an exterior of  
15 the vesicle. Typically, the exterior surface of the wall is formed by amphiphilic molecules oriented such that their hydrophilic regions are in contact with water, the solvent in the aqueous solution. The interior surface of the wall may be formed by amphiphilic molecules oriented such that their hydrophilic regions are in contact with water present in the interior of the vesicle, or the interior surface of the wall may be  
20 formed by amphiphilic molecules oriented such that their hydrophobic regions are in contact with hydrophobic materials present in the interior of the vesicle.

The amphiphilic molecules in the wall will tend to form layers, and therefore, the wall may comprise one or more layers of amphiphilic molecules. If the wall consists of one layer, it may be referred to as a “unilayer membrane” or “monolayer membrane.” If  
25 the wall consists of two layers, it may be referred to as a “bilayer membrane.” Walls with more than two layers, up to any number of layers, are also within the scope of the present invention.

The vesicle may be referred to herein as a “buckysome.”

In one embodiment, the vesicle wall is a bilayer membrane. The bilayer  
30 membrane comprises two layers, an interior layer formed from the amphiphilic fullerene

and other amphiphilic compound or compounds, if any, wherein substantially all the amphiphilic fullerene and other amphiphilic molecules are oriented with their hydrophobic portions toward the exterior layer, and an exterior layer formed from the amphiphilic fullerene and other amphiphilic compound or compounds, if any, wherein substantially all the amphiphilic fullerene and other amphiphilic molecules are oriented with their hydrophobic portions toward the interior layer. As a result, the hydrophilic portions of substantially all molecules of each of the interior and exterior layers are oriented towards aqueous solvent in the vesicle interior or exterior to the vesicle.

For further details on the amphiphilic fullerenes and vesicles made therefrom, see  
10 Hirsch et al., U.S. Pat. Appl. 10/367,646, filed February 14, 2003, for “Use of  
Bucksome or Carbon Nanotube for Drug Delivery,” which is incorporated herein by  
reference.

The method can be used to treat one or more dermatological conditions,  
including, but not limited to, sunburn, aging, hair loss, psoriasis, acne, or smoker’s face.

15 We have discovered that substituted fullerenes have antioxidant properties and  
thus can inhibit ROS damage to cells exposed to ROS or cells wherein ROS are  
generated.

It is known that fullerenes are essentially non-toxic and non-allergenic, including  
in topical skin applications. For example, see Huczko et al., *Fullerene Sci. Tech.*  
20 7(5):935-939 (1999).

25 In one embodiment, the substituted fullerene is a component of a composition  
also comprising a carrier. The carrier can be any material or plurality of materials which  
can form a composition with the substituted fullerene. The particular carrier can be  
selected by the skilled artisan in view of the intended use of the composition and the  
properties of the substituted fullerene, among other parameters apparent in light of the  
present disclosure.

30 The composition can comprise the substituted fullerene in any form. In one  
embodiment, the substituted fullerene is dispersed or dissolved in an aqueous solution  
suitable for topical administration. In another embodiment, the substituted fullerene is  
dispersed or dissolved in an aqueous solution suitable for subcutaneous administration.

In a further embodiment, the substituted fullerene is dispersed or dissolved in a water-in-oil emulsion, a microemulsion, a multiple emulsion, a gel, or a fluorocarbon emulsion.

Non-limiting examples of particular carriers and particular compositions follow.

5 In one embodiment, the carrier is water, and the composition is an aqueous solution comprising water and the substituted fullerene. The composition can further comprise solutes, such as salts, acids, bases, or mixtures thereof, among others. The composition can also comprise a surfactant, an emulsifier, or another compound capable of improving the solubility of the substituted fullerene in water.

10 In one embodiment, the carrier is a polar organic solvent, and the composition is a polar organic solution comprising the polar organic solvent and the substituted fullerene. “Polar” has its standard meaning in the chemical arts of describing a molecule that has a permanent electric dipole. A polar molecule can but need not have one or more positive, negative, or both charges. Examples of polar organic solvents include, but are not limited to, methanol, ethanol, formate, acrylate, or mixtures thereof, among others. The 15 composition can further comprise solutes, such as salts, among others. The composition can also comprise a surfactant, an emulsifier, or another compound capable of improving the solubility of the substituted fullerene in the polar organic solvent.

20 In one embodiment, the carrier is an apolar organic solvent, and the composition is an apolar organic solution comprising the apolar organic solvent and the substituted fullerene. “Apolar” has its standard meaning in the chemical arts of describing a molecule that does not have a permanent electric dipole. Examples of apolar organic solvents include, but are not limited to, hexane, cyclohexane, octane, toluene, benzene, or mixtures thereof, among others. The composition can further comprise solutes, such as apolar molecules, among others. The composition can also comprise a surfactant, an 25 emulsifier, or another compound capable of improving the solubility of the substituted fullerene in the apolar organic solvent. In one embodiment, the composition is a water-in-oil emulsion, wherein the substituted fullerene is dissolved in water and water is emulsified into a continuous phase comprising one or more apolar organic solvents.

30 In one embodiment, the carrier is a mixture of water and other solvents. In one embodiment, the carrier can comprise one or more of dimethicone, water, urea, mineral

oil, sodium lactate, polyglyceryl-3 diisostearate, ceresin, glycerin, octyldodecanol, polyglyceryl-2 dipolyhydroxystearate, isopropyl stearate, panthenol, magnesium sulfate, bisabolol, lactic acid, lanolin alcohol, or benzyl alcohol, among others.

In one embodiment, the composition has a creamy consistency suitable for  
5 packaging in a squeezable plastic container. In one embodiment, the composition has a lotion consistency suitable for packaging in a squeezable plastic container. In one embodiment, the composition has an ointment-like consistency suitable for packaging in a squeezable plastic container. In one embodiment, the composition has a liquid consistency suitable for packaging in a non-squeezable container. A non-squeezable  
10 container can be fabricated from one or more of plastic, glass, metal, ceramic, or other compounds. A non-squeezable container can be fabricated with a flow-type cap or a pump-type dispenser.

Other carriers will be apparent to the skilled artisan having the benefit of the present disclosure.

15 In one embodiment, the carrier is a pharmaceutically-acceptable carrier. By “pharmaceutically-acceptable” is meant that the carrier is suitable for use in medicaments intended for administration to a mammal. Parameters which may be considered to determine the pharmaceutical acceptability of a carrier can include, but are not limited to, the toxicity of the carrier, the interaction between the substituted fullerene and the carrier,  
20 the approval by a regulatory body of the carrier for use in medicaments, or two or more of the foregoing, among others. In one embodiment, further components of the composition are pharmaceutically acceptable.

25 In addition to the substituted fullerene and the carrier, and further components described above, the composition can also further comprise other compounds, such as preservatives, adjuvants, excipients, binders, diluents, surfactants, or other adjuncts other agents capable of ameliorating one or more diseases, or mixtures thereof, among others. In one embodiment, the other compounds are pharmaceutically acceptable.

30 The concentration of the substituted fullerene in the composition can vary, depending on the carrier and other parameters apparent to the skilled artisan having the benefit of the present disclosure. A typical composition can comprise from about 0.01

wt% to about 5 wt% substituted fullerene. In one embodiment, the composition can comprise from about 0.1 wt% to about 0.5 wt% substituted fullerene. The concentration of other components of the composition can also vary along the same lines. Generally, the balance of the composition will comprise the carrier and other compounds.

5        The composition can be administered to any portion of the skin of the mammal. The composition can be administered after diagnosis of a dermatological condition (i.e., the mammal is afflicted with the dermatological condition) or prophylactically to minimize the likelihood of acquiring a dermatological condition, its severity, or both (i.e., the mammal is potentially afflicted with the dermatological condition). An example of a  
10      mammal potentially afflicted with a dermatological condition is an *H. sapiens* intending to be exposed to sunlight on a day with few clouds, especially such a day during summer, at low latitude, at high altitude, or a combination thereof, in which case a dermatological condition with which the *H. sapiens* may potentially be afflicted is sunburn.

15        The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the  
20      present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

25        A human test subject exposed two portions of his skin to daytime sunlight at about 22° north latitude for a duration of about three hours in the early afternoon of day 0. As a result, he presented with a sunburn, as shown in Figure 1, day 0. Within a few minutes after the photograph of Figure 1, day 0 was taken, a composition comprising a substituted fullerene having the structure shown in Figure 8B at 1% in an inorganic cream  
30      base comprising dimethicone, water, urea, mineral oil, sodium lactate, polyglyceryl-3

diisostearate, ceresin, glycerin, octyldodecanol, polyglyceryl-2 dipolyhydroxystearate, isopropyl stearate, panthenol, magnesium sulfate, bisabolol, lactic acid, lanolin alcohol, and benzyl alcohol (commercially available under the trade name Eucerin, Beiersdorf, Inc., Wilton, CT) was administered in the form of a topical ointment to the portion of the 5 subject's skin inside the notional box shown in Figure 1, day 0. The administration regimen involved two applications per day for two days. The outside control portion received no treatment.

After about 48 hrs, the same two portions of the subject's skin were 10 photographed, and this photograph is shown as Figure 1, day 2. The control portion of the skin showed peeling, blistering, or both, as can be seen in Figure 1, day 2. In contrast, the treated portion of the subject's skin showed very little peeling or blistering.

Example 2

Superoxide radicals were generated by employing the xanthine/xanthine 15 oxidase/cytochrome c system. The reaction was initiated by the addition of xanthine oxidase ( $7.5 \times 10^{-3}$  units) to the incubation mixture and the reaction was followed in terms of the reduction of cytochrome c and the corresponding increase in the absorbance at 550 nm. The reduction of ferricytochrome c into ferrocytochrome c was determined using the molar absorption coefficients of  $9 \text{ mmol}^{-1}\text{cm}^{-1}$  and  $27.7 \text{ mmol}^{-1}\text{cm}^{-1}$  for the 20 oxidized and reduced forms, respectively. All assays were performed at room temperature. The incubation mixture consisted of 50 mM potassium phosphate, 0.1 mM EDTA, 0.01 mM cytochrome c, and 0.05 mM xanthine, along with the indicated concentration of antioxidant. A total volume of 3 mL was used in each experiment.

Various substituted fullerenes, both those known in the art and those reported 25 herein, were tested, as shown in Figure 11. Trolox, a known non-fullerene antioxidant, was tested as a comparative example. A negative control (without antioxidant, not shown) was run to establish a baseline for the reduction of cytochrome c. The compounds and their IC<sub>50</sub> values are given in Figure 11. Comparative compounds are indicated with the notation "(Comparative)."

Of the comparative compounds, DF-1 had the lowest IC<sub>50</sub>, 102 µm. However, many of the compounds of the present invention had much lower IC<sub>50</sub> values, indicating higher antioxidant properties.

5 All of the compositions and the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of particular embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and the methods and in the steps or in the sequence of  
10 steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope  
15 and concept of the invention as defined by the appended claims.